

Comparative Biochemistry of Hemoglobins

VI. Isolation of the So-called Soluble Tryptic Peptides from the α -Polypeptide Chain of *Macaca mulatta* Monkey Hemoglobin and their Amino Acid Compositions

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Received for publication February 10, 1966

The α -polypeptide chain of *Macaca mulatta* monkey hemoglobin was isolated by the countercurrent distribution method with a water - 0.08% trichloroacetic acid in sec. - butanol-propionic acid (11.0 : 8.7 : 1.5) system and was digested with trypsin, and the tryptic peptides soluble at pH 6.4 were separated and purified from the digest by column chromatography using Dowex 1 \times 2 as the adsorbent and a mixture of organic solvents as the developer, and by paper chromatography to investigate their amino acid compositions.

Among them, eleven tryptic peptides and one amino acid were included, and their comparison with those from the α -chain of human adult hemoglobin has revealed that three of the peptides from the *Macaca mulatta* monkey have different amino acid compositions from the corresponding peptides from the human adult, and that the sequence of 101 amino acids contained in all the peptides from the *Macaca mulatta* monkey has at least five amino acid exchanges with that of the corresponding peptides from the human adult.

INTRODUCTION

Our laboratory has had interest in the evolutionary problem of the hemoglobin molecule, and has been studying the comparative biochemistry on hemoglobins from various species of animals. Originally, the problem of evolution of living matters has been studied by a comparative biological approach based on mainly the form and habit of living matters. However, as every living body is composed of chemical substances, comparative biochemical studies on its components would be necessary for the purpose of elucidation of the problem of biological evolution. Recently, the development of protein researches has demo-

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nstrated interspecies heterogeneity in the primary structure of various proteins. Based on this fact, ANFINSEN'S²³⁾ researches aimed to compare the structures of various proteins, and to conjecture the evolutionary process of the gene which controls the biosynthesis of the proteins.

Hemoglobin, a chromoprotein with protoheme, is very widely distributed in the animal kingdom, and the primary structures of the α -,^{3),9)} β -³⁾ and γ -¹⁷⁾ polypeptide chains of human hemoglobin and the primary structures of the α -¹¹⁾ and β -¹⁹⁾ polypeptide chains of horse hemoglobin have been already determined. ZUCKERKANDL and PAULING²⁵⁾, attempting a genetic study on the previously reported fact that 17 amino acid exchanges are observed between the α -chain of human adult hemoglobin and that of horse hemoglobin, have assumed that it will take 14.5×10^6 years for one amino acid to exchange with another, and supposed the time when the α -, β - and γ -polypeptide chains of human hemoglobin differentiated from each other.

On the other hand, as the monkey is believed to be the most closely related animal to man in the evolutionary process, the difference between monkey and human hemoglobins is a very interesting matter for study. Concerning the resemblance between these two kinds of hemoglobin, various reports have been published initiated by an immunological comparative study of SCHAPIRA and KRJH¹⁶⁾, but few reports have shown any clear differences between them. In relatively recent years, ZUCKERKANDL et al²⁴⁾ compared the tryptic peptides soluble at pH 6.4 obtained from tryptic digests of *Macaca mulatta* monkey hemoglobin with those of human hemoglobin by the fingerprinting method, and recognized some differences between them. MÄSIAR¹⁰⁾, however, performed similar experiments, but could not recognize any differences in the tryptic peptides soluble at pH 6.4. MATSUDA and MAITA¹²⁾ also have recognized by their analysis of the N-terminal amino acids by the DNP-method that *Macaca mulatta* monkey hemoglobin, as well as human adult hemoglobin, has 2 moles of Val-Leu and 2 moles of Val-His-Leu N-termini in 1 mole of hemoglobin. Then, SHIKAYA¹⁸⁾, in order to compare *Macaca mulatta* monkey hemoglobin with human hemoglobin, has examined those two kinds of hemoglobin by agar-gel electrophoresis, cellulose-acetate membrane electrophoresis, CM-cellulose column chromatography and alkali denaturation method and by the fingerprinting method on their tryptic peptides, and has confirmed that, though they have a close resemblance to each other, a slight but clear structural difference is existent between them. BUETTNER and HILL⁴⁾, concerning the α -polypeptide chain of *Lemur fulvus* hemoglobin which has less close evolutionary relation to man than the *Macaca mulatta* monkey, has isolated 10 tryptic peptides from its tryptic hydrolysate by the fingerprinting method and column chromatography, examined the compositions of 101 amino acids in them, and recognized that there are 6 amino acid

exchanges between these peptides and the peptides from the α -polypeptide chain of human adult hemoglobin.

The present author, isolated and purified 11 peptides and 1 amino acid soluble at pH 6.4 from the tryptic digest of the α -polypeptide chain from *Macaca mulatta* monkey hemoglobin by column chromatography using Dowex 1 \times 2 as the adsorbent and a mixture of organic solvents as the developer and by paper chromatography with a mixture of organic solvents, analyzed their amino acid compositions, and compared them with the tryptic peptides obtained from the α -chain of human hemoglobin.

MATERIALS AND METHODS

1) *Preparation of the α -Polypeptide Chain*

Macaca mulatta monkey adult hemoglobin was separated and purified by a modified method of DRABKIN's⁵⁾ from the blood obtained from the abdominal aorta of a 4-year-old male *Macaca mulatta* monkey weighing 4 kg. From the hemoglobin obtained, globin was prepared by the removal of the heme according to TEALE's methyl-ethyl ketone method²²⁾. The isolation of the α - and β -polypeptide chain from the globin was carried out according to the method of MATSUDA et al¹³⁾, a modified form of the countercurrent distribution method using a water-0.08%-trichloroacetic acid in sec. -butanol-propionic acid (11.0:8.7:1.5) system which was employed by HILSCHMANN and CRAIG⁶⁾ for the purification of Bence-Jones protein. The α -polypeptide chain obtained in this way was dialyzed against deionized water and then lyophilized.

2) *Digestion of the α -Polypeptide Chain with Trypsin*

Before tryptic digestion, the α -polypeptide chain was dissolved in 8M urea solution by a concentration of 1% and denatured in a water-bath at 60°C for 45 min. After cooling, the solution was dialyzed against deionized water to remove urea. Trypsin (Warthington Biochemical Company, twice crystallized) was dissolved in 1/16 N HCl by a concentration of 1%, and kept at 37°C for 12 hr to remove the activity of a contaminating chymotrypsin-like substance according to the method of REDFIELD and ANFINSEN¹⁴⁾. Five hundred milligrams of the urea-denatured α -polypeptide chain were suspended in 50cc of deionized water in a bottle kept at 37°C and the suspension was adjusted to pH 8.0 by adding 0.1N NaOH as stirring the suspension violently. To the suspension, 10mg of trypsin contained in 1cc of the above-mentioned 1/16 N HCl solution were added and digestion was performed at 37°C. During the digestion the suspension was kept at pH 8.0 by adding 0.1 N NaOH. The quantities of 0.1 N NaOH added were recorded every hour. After 2hr, additional 5mg of trypsin were added

and the digestion was continued for 2 hr more. The reaction was stopped by adding 10% acetic acid.

3) *Isolation of the So-called Soluble Tryptic Peptides of the α -Polypeptide Chain.*

When the trypsin digest was adjusted to pH 6.4, it yielded insoluble precipitate, so-called "core". This solution, after being stirred violently for 30 min, was made sure to be pH 6.4 again and allowed to stand in the cold room overnight. The next morning this precipitate was separated by centrifuging (3,000r. p. m., 20 min) and the peptides soluble at pH 6.4 in the supernatant were obtained. This solution was concentrated in vacuum in a rotary evaporator and then lyophilized.

4) *Column Chromatography of the Tryptic Peptides on Dowex 1 \times 2*

One pound of Dowex 1 \times 2 (Dow Chemical Company, 200–400 mesh, Cl-form) was suspended in three volumes of deionized water and after being stirred for 20 min and allowed to stand 4 hr, the floating particles were discarded by decantation. This treatment was repeated three times to make even the grains of the resin. This resin was placed on a glass-filter and washed with 3 L of acetone and 3 L of deionized water successively. Then the resin was suspended in three volumes of 1N NH_4OH in a beaker, and allowed to stand for 1 hr being stirred sometimes. After that it was placed on a glass-filter to remove NH_4OH by filtration and washed with deionized water till the filtrate became neutral. This resin in the OH-form was converted to the acetic acid form by being suspended in three volumes of acetic acid in a beaker and allowed to stand for 1 hr being stirred sometimes. The acetic acid was removed by a glass-filter and after that the resin was washed with deionized water till the pH of the filtrate became about 5.5.

The resin in the acetic acid form thus obtained was suspended in three volumes of the starting buffer and equilibrated as being stirred. Air was removed from the equilibrated resin and the resin was packed into a column (2.0 \times 150cm) which was kept at 37°C. After the adjustment of the flow rate, the resin in the column was equilibrated with additional 3 L of the strating buffer. The sample (the peptides corresponding 500mg of the α -chain) was dissolved in 40cc of deionized water and put on the column. The elution was carried out at 37°C and a flow rate of 100cc/hr with the starting buffer, 1% pyridine–1% collidine acetate buffer, pH 8.5, until fraction No. 25 and after that the gradient elution was employed. Wth 1.5 L of 1% pyridine 1% lutidine–1% picoline acetate buffer, pH 7.5 in the mixing coamber, elution was performed by pH gradient supplying 0.075 N acetic between acid fractions No. 26 and No. 230 and 1.0 N acetic acid between fractions No. 231 and No. 270. Finally, the column was washed with glacial acetic acid. All the organic solvents used were purified by distillation,

and the deionized water was boiled to remove carbon dioxide and then degassed thoroughly in vacuum. The eluate was collected in 20cc fractions by the fraction collector. A 0.2cc portion each of the fractions, after alkali hydrolysis, was tested on the ninhydrin reaction according to the method of YEMM and COKING²³⁾. To the 0.2cc portion from each tube, which was taken in a hard-glass tube, 1.0cc of 2.5 N NaOH was added and the tube was capped with aluminum foil and hydrolyzed in an oil-bath at 95°C for 2.5 hr. After cooling, 1.0cc of 30% acetic acid was added to each tube to make the pH of the solution about 5.0. To the solution 0.5cc of 0.2M citrate buffer (pH 5.0) and 1.2cc of ninhydrin-KCN solution were added and the tube was capped again with aluminum foil and heated at 100°C in an oil-bath for 15 min. After the tube was immediately cooled with flowing water, the solution was diluted with 3cc of 60% ethanol and measured at an absorbancy of 570 $m\mu$. The fraction of the nynthrin-positive peaks were collected, and after concentration in vacuum in a rotary evaporator under 30°C, was lyophilized. These fractions containing peptides were dissolved in 3.0cc of deionized water and stored in the freezer.

5) *Identification of Peptides by Paper Chromatography*

Identification of the peptides was performed by paper chromatography using Toyo filter paper, No. 50, by the descending method with a n-butanol-acetic acid-water (4:1:5) system at a constant temperature (24–25°C). In identification, 30 μ l each of the fractions were applied to the paper. The peptides on the chromatogram were detected by spraying 0.2% ninhydrin-butanol solution on the paper and heating the paper with an iron to color. For the specific amino acid color tests were employed EHRLICH's reaction²⁰⁾, PAULY's reaction²¹⁾, nitrosonaphthol reaction¹⁾, and SAKAGUCHI's reaction⁸⁾. In this case, for example, three paper chromatograms were prepared. One was tested primarily by the ninhydrin color reaction and subsequently by EHRLICH's reaction and then Pauly's reaction. The other two were tested first by the ninhydrin reaction to recognize spots and secondarily by the nitrosonaphthol reaction and SAKAGUCHI's reaction, respectively.

6) *Purification of Peptide by Paper Chromatography*

Purification of the peptides by paper chromatography was carried out by almost the same method described above for the identification of the peptides. For the color reaction, 0.02% ninhydrin-butanol solution was used and these spots of peptides, which were lightly colored, were cut out. The ninhydrin on the spots was washed off with acetone, and the spots were dried in air. In a closed chamber, the peptides were eluted from the respective spots which were attached at the tip of the filter-paper strip hung from the petri dish filled with 5%

acetic acid. The eluate was concentrated to dryness in vacuum in a rotary evaporator under 35°C.

7) Amino Acid Analysis of the Peptides

Each of the peptides purified and concentrated to dryness was dissolved in 4cc of constant boiling HCl twice distilled, and hydrolyzed at 105°C for 20 hr in a sealed tube. The hydrolysate was concentrated to dryness in vacuum in a rotary evaporator, and after addition of small volume of deionized water, was concentrated to dryness again to remove HCl as perfectly as possible. The amino acids were analyzed by the Hitachi KLA-2 amino acid analyzer. The acidic and neutral amino acids were analyzed with a 50cm column with a buffer change time of 2.5 hr (from pH 3.25 to pH 4.25), and the analysis was completed in 6 hr. The basic amino acids were analyzed with a 7.5cm column, and the analysis were performed for 3 hr.

RESULTS AND DISCUSSION

The α - and β -polypeptide chains were isolated from 0.5g of globin by the countercurrent distribution method with 150 transfers, and the result was shown in Fig.1. Each of the α - and β -chains isolated was assured to be pure by cellulose-acetate membrane electrophoresis, and the analysis of the N-terminal peptides of the chains by the DNP-method

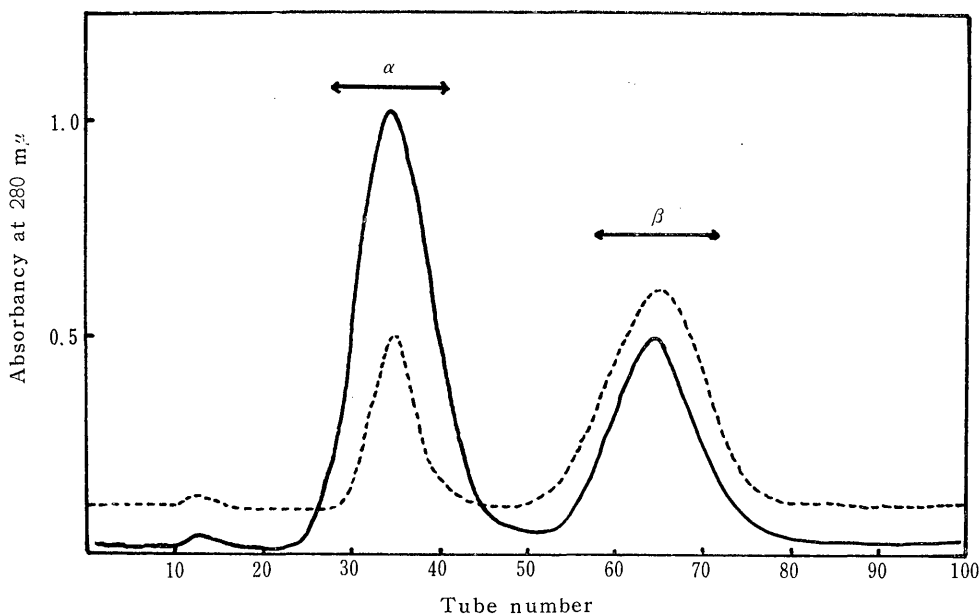


Fig. 1. Countercurrent distribution of the globin from *Macaca mulatta* monkey for 150 transfers, 10cc in each phase. The sample (0.5g) was initially placed in tubes No. 3-7, —•— lower phase, upper phase

revealed only DNP-Val-Leu for the α -chain and only DNP-Val-His-Leu for the β -chain. The α -chain isolated in this way was first digested with trypsin. The digestion was carried out at an enzyme concentration of 2%, pH 8.0 and 37°C. In 45 min after the start of the digestion the reaction mixture became almost transparent. The course of the reaction was pursued by the alkali uptake, and the result was shown in Fig.2. Till 1 $\frac{2}{3}$ hr after the start of the digestion, the alkali uptake

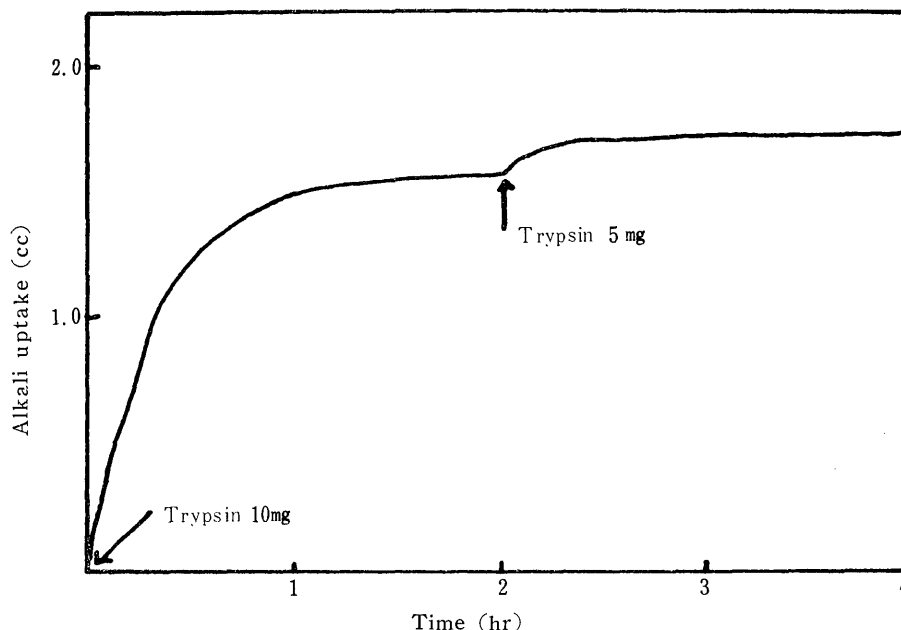


Fig. 2. Course of the tryptic digestion of the α -chain of *Macaca mulatta* monkey hemoglobin

increased, but after that, the uptake became small. To make the digestion complete, trypsin was added again after 2 hr so that it may become 3% at the whole volume, but in the continued digestion the increase of the alkali uptake stopped in about 30 min.

Among the peptides obtained from the tryptic digestion of hemoglobin, there are so-called "core" which is insoluble and peptides which are soluble at pH 6.4. Therefore, "core" was precipitated by adjusting the tryptic digest to pH 6.4 and removed by centrifuging. For the fractionation of peptides, there are several methods such as paper chromatography, paper electrophoresis, fingerprinting method combining the preceding two methods, column chromatography, and countercurrent distribution method. In the present experiment, column chromatography using Dowex 1 \times 2 as the adsorbent and a mixture of organic solvents as the developer was employed for fractionation, and paper chromatography was used for isolation and identification.

The fractionation of peptides by column chromatography was descri-

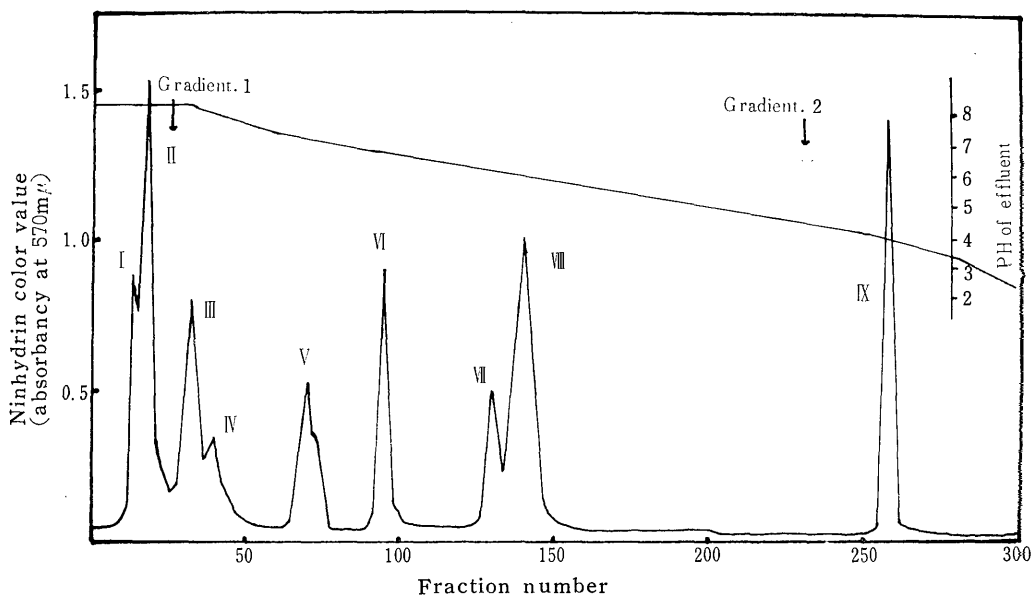


Fig. 3. Chromatography on Dowex 1×2 of the soluble tryptic peptides from the α -chain of *Macaca mulatta* monkey hemoglobin

bed in detail on human hemoglobin by RUDLOFF and BRAUNITZER¹⁵). It is said to be especially suitable to the isolation of peptides containing many heterocyclic amino acids. Modifying this method, the author isolated the so-called soluble tryptic peptides from the α -polypeptide chain of *Macaca mulatta* monkey adult hemoglobin. Fig. 3 shows the elution pattern of column chromatography. As observed in this figure, 9 peaks appeared. The comparison of this with the result for the soluble tryptic peptides from the α -polypeptide chain of human adult hemoglobin obtained by HILSE and BRAUNITZER⁷), though the conditions of elution was somewhat different, gave closely resembling appearances of peaks between them. The moderation of the pH gradient in this experiment gave a better separation of peaks VI, VII and VIII. To make sure of the purity of the peptides present in the peaks, the eluate from each peak was collected, and after lyophilization, dissolved in hot water and subjected to paper chromatography. The paper chromatography was carried out with a n-butanol-acetic acid-water (4:1:5) system, by the descending method because the ascending method gives remarkable disorder compared with the descending method. Fig. 4 gives a paper chromatogram with the results of the ninhydrin reaction and specific amino acid color reactions.

Spot I-a seems to be the same as spot II-a. Spot II-b is 0.17 in Rf Leu, quite similar to lysine. Rf Leu of a peptide is its Rf value calculated setting the Rf value of leucine at 1.0. In the fraction of peak II, 4 main spots and 2 minor spots were observed. But these spots were observed to separate completely by this chromatography.

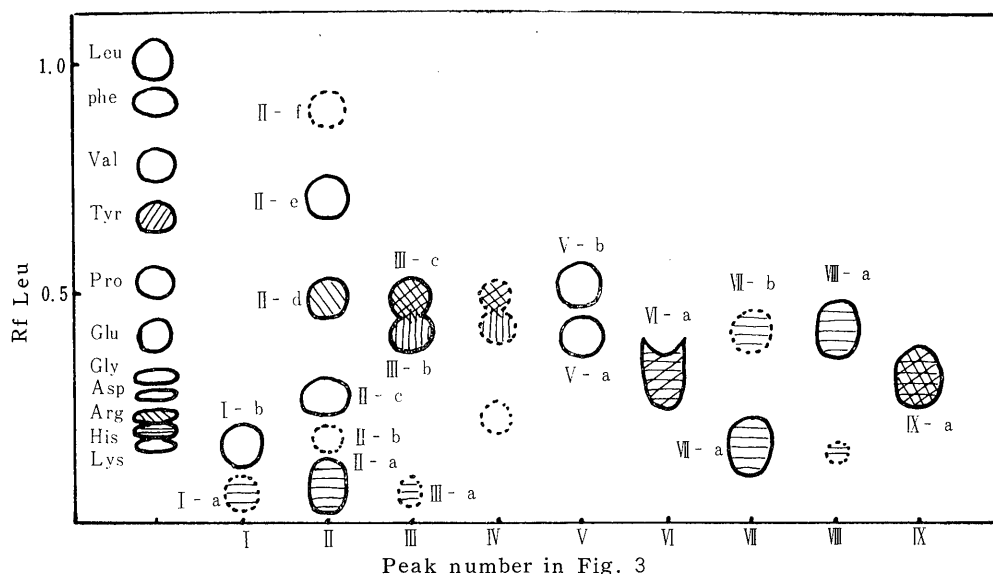


Fig. 4. Paper chromatogram of the soluble tryptic peptides from the α -chain of *Macaca mulatta* monkey hemoglobin

His Arg Tyr Trp

And II-e and II-f were demonstrated to have the similar amino acid compositions to each other by the amino acid analysis performed later.

As they are peptides containing methionine, the difference between them on the paper chromatogram seems to be due to the denaturation of the methionine. In the fraction of peak III, one main spot and one minor spot were observed by ninhydrin reaction. The lower III-a seems to have been the same as II-a.

As the upper main spot was distinguished to consist of an arginine- and tyrosine-positive upper half and a tryptophan-positive lower half, the halves are designated III-b and III-c. This main spot was tried to separate into an arginine- and tyrosine-positive spot and a tryptophan-positive spot by high-voltage filter-paper electrophoresis with pyridine-acetate buffer, pH 6.4, but it could not be achieved. The fraction of peak IV is almost the same as the fraction of peak III. In the fractions of peaks V, VI, VII, VIII and IX, were observed 2, 1, 2, 2 and 1 spots, respectively, which were all perfectly separated by this paper chromatography. Each of the peptides purified in this way was hydrolyzed with constant boiling HCl solution at 105°C for 20 hr, and its amino acid composition was examined by the automatic amino acid analyzer (Hitachi KLA-2). The molar ratios of the amino acids from the peptides are shown in Table 1. The value for every amino acid is not corrected for the decomposition during hydrolysis. Spot I-b (R_f Leu = 0.17) is consistent with lysine in paper chromatography, and the

Table 1

Amino acid composition of the soluble tryptic peptides from the α -chain of *Macaca mulatta* monkey hemoglobin

	I -b	II -a	II -c	II -d	II -e	III -b	III -c	V -a	V -b	VI -a	VIII -a	IX -a
Lys	1.00	1.08	1.10		1.02	1.01		1.05	1.02	1.13	1.13	
His		1.04								1.94	3.01	0.99
Arg				1.07			1.00					1.07
Met-O ₂					+						+	
Asp			1.11					2.13	1.07	1.15	4.13	
Thr					1.87					0.85	0.93	
Ser			0.80		0.92				0.92	1.89	1.80	
Glu										1.17	0.97	3.08
Pro					1.17			0.96	0.99	0.99	1.09	
Gly		1.88				1.03				1.16	1.10	4.14
Ala						1.96			1.13	1.13	6.03	3.02
Val			0.99					1.87	0.93	0.88	2.88	0.89
Leu				0.93	1.08				0.94	1.00	4.93	1.09
Tyr							0.75			0.78		0.72
Phe					1.94			0.99		1.94		
Try						+						

amino acid analysis after hydrolysis also gave lysine alone. Therefore, this may correspond to the so-called H α T8 peptide from the α -polypeptide chain of human adult hemoglobin. As peptide II -a (Rf Leu=0.07) is the same as I -a and III -a and its amino acid composition is quite similar to that of H α T7, it is a peptide representing Gly-His-Gly-Lys (57-60) which is called basic center of the α -chain of hemoglobin. Peptide II -c (Rf Leu=0.26) composed of 4 amino acids, is presumed to correspond to H α T2, but as the result of analysis revealed one mole of serine instead of threonine which was observed in human hemoglobin, an exchange of threonine with serine is considered to have occurred between human and *Macaca mulatta* monkey hemoglobins.

As the result of the amino acid analysis of peptide II -d (Rf Leu=0.49), arginine and leucine were detected by equal moles, and it is thought to correspond to H α T10. Peptides II -e and II -f both contained methionine and were quite similar to H α T5 in the composition of the other amino acids, and only owing to the denaturation of methionine, the spot is thought to appear sometimes at II -e or other times at II -f. Spot III -b, c (Rf Leu =0.40-0.48) consists of 2 peptides, which could

not be separated completely from each other. This spot, the upper half being arginine- and tyrosine-positive and the lower half tryptophan-positive, was eluted and its amino acids were analyzed. The result showed 0.371 μ mole of lysine, 0.549 μ mole of arginine, 0.381 μ mole of glycine, 0.721 μ mole of alanine and 0.412 μ mole of tyrosine. These were assumed to be two peptides correspondent to H α T3 (Ala-Ala-Try-Gly-Lys) and H α T14 (Tyr-Arg), and the above results of amino acid analysis were divided into two groups: (1) lysine, alanine, and glycine, and (2) arginine and tyrosine, the molar ratios of which were examined. In the former group, lysine was 1.01, glycine 1.03 and alanine 1.96, and as the tryptophan reaction on the paper chromatogram revealed a deeply-colored spot a little lower than the position of tyrosine and arginine, tryptophan should be added in this peptide. From this fact, peptide III-b is considered to have quite a similar amino acid composition to H α T3. In the latter group, arginine was 1.00, and tyrosine 0.75, and this peptide corresponds to the C-terminal peptide H α T14 of the α -chain of human hemoglobin. The peptides in the fraction of peak IV were the same as III-b and III-c, below which was observed a very light spot. The amino acids of the spot were analyzed, but they are so little in amount that no good determination value could be obtained. This may not be a significant tryptic peptide. Peptides V-a (Rf Leu=0.42) and V-b (Rf Leu=0.51) didn't show any specific reactions and their amino acid compositions were the same as those of H α T11 and H α T1, respectively. Assuming V-b to correspond to H α T1, this fact is consistent with the result of MATSUDA and MAITA¹²⁾ that the α -chain of *Macaca Mulatta* hemoglobin begins with Val-Leu which was obtained from the analysis of the N-terminal amino acids by the DNP-method.

From the fact that peptide VI-a (Rf Leu=0.31) is proved to be positive in histidine and tyrosine reactions on the paper chromatogram, and is eluted near the neutral peptides in column chromatography on Dowex 1 \times 2, and that its amino acid composition is quite consistent with that of H α T6, it is conjectured that α T6 consisting of 16 amino acids is quite similar in both human and *Macaca mulatta* monkey hemoglobins. Peptide VIII-a (Rf Leu=0.42) consists of 29 amino acids, 2 moles less of aspartic acid and 1 mole less of alanine and 1 mole each more of glutamic acid, glycine and leucine than H α T9. From the fact, it is thought that this peptide from *Macaca mulatta* monkey hemoglobin has at least 3 amino acid exchanges with corresponding α T9 from human hemoglobin. Peptides VII-a (Rf Leu=0.15) and VII-b (Rf Leu=0.41) were observed to have the same relationship as observed between II-e and II-f. And these two peptides were proved to have only 1 mole more of lysine than peptide VIII-b as the result of amino acid analysis.

These correspond to H α T8-9 which is indecomposed Lys-Lys (65-

Table 2

Comparison between the amino acid compositions of the tryptic peptides from the α -chain of human and *Macaca mulatta* monkey hemoglobins

peptide no	αT_1		αT_2		αT_3		αT_4		αT_5		αT_6		αT_7		αT_8		αT_9		αT_{10}		αT_{11}		αT_{14}	
	M	H	M	H	M	H	M	H	M	H	M	H	M	H	M	H	M	H	M	H	M	H	M	H
Lys	1	1	1	1	1	1			1	1	1	1	1	1	1	1	1	1			1	1		
His							1					2	2	1	1			3	3					
Arg							1	1												1	1		1	1
Asp	1	1	1	1							1	1					④	⑥			2	2		
Thr				①					2	2	1	1					1	1						
Ser	1	1	①						1	1	2	2					3	2						
Glu							3	3			1	1					①							
Pro	1	1							1	1	1	1					1	1			1	1		
Gly					1	1	④	③			1	1	2	2			①							
Ala	1	1			2	2	③	④			1	1					⑥	⑦						
Val	1	1	1	1			1	1			1	1					3	3			2	2		
Met									1	1							1	1						
Leu	1	1					1	1	1	1	1	1					⑤	④	1	1				
Tyr							1	1			1	1											1	1
Phe									2	2	2	2									1	1		
Try					1	1																		
Total	7	7	4	4	5	5	15	15	9	9	16	16	4	4	1	1	29	29	2	2	7	7	2	2

66) which was obtained also when the α -chain of human hemoglobin was digested with trypsin. Peptide α -a (Rf Leu=0.32), an acidic peptide which is eluted at near pH 4.0 in Dowex 1 \times 2 column chromatography, could be isolated almost purely by only column chromatography, but for amino acid analysis it was used after another purification by paper chromatography. The comparison of its amino acid composition with that of H α T4, demonstrated that it had 1 mole less of alanine and 1 mole more of glycine than H α T4 with all the same other amino acids. From the fact, it is supposed that one alanine of H α T4 from human hemoglobin exchanges with glycine in the peptide from *Macaca mulatta* monkey hemoglobin. Table 2 gives the comparison between the amino acid compositions of the tryptic peptides from the α -polypeptide chain of human hemoglobin and those of their corresponding peptides from *Macaca mulatta* monkey hemoglobin.

As shown in the table, between *Macaca mulatta* monkey adult hemoglobin and human adult hemoglobin, at least 5 exchanges were recognized in the primary structure containing 101 amino acids of the peptides soluble at pH 6.4 which were obtained when the α -polypeptide chain was digested with trypsin. These amino acid exchanges are

supposed to occur between threonine and serine; alanine and glycine; aspartic acid and glutamic acid; and aspartic acid and leucine. These amino acid exchanges are observed between human adult hemoglobin and horse hemoglobin.

CONCLUSION

Macaca mulatta monkey adult hemoglobin has been purified and the α -polypeptide chain in it has been isolated by the countercurrent distribution method and hydrolyzed with trypsin. Then the tryptic peptides soluble at pH 6.4 have been isolated and purified from the hydrolysate by column chromatography on Dowex 1 \times 2, and by paper chromatography. The peptides obtained in this way have been compared with those obtained from human adult hemoglobin, in their amino acid compositions, which enables us to suppose that there are at least 5 amino acid exchanges in the sequence of 101 amino acids between these two kinds of hemoglobin.

ACKNOWLEDGEMENTS

The author wishes to express his cordial gratitude to Prof. Dr. MATSUDA who gave him constant and useful guidance and encouragement and also to Dr. T. MAEKAWA who gave him kind advice during his work.

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